

# GRAM STAINING

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# INTRODUCTION.

- 1884....Danish physician Hans Christian Gram.
- Gram +ve □ stain blue(purple)
- Gram -ve □ red (pink).

# PRINCIPLE.

- Difference in staining reactions is due to difference in cell wall of gram +ve & gram -ve bacteria.
- Gram +ve □ Thick layer of peptidoglycan holds basic dye and resists decolorization with acetone or ethanol.

# METHOD.

- Preparation of smear.
- Fixation.
- Staining.

# REQUIREMENTS.

- Crystal violet stain (dye) □ Stains cells blue/purple,
- Lugol's iodine □ Mordant (To reinforce primary color),
- Acetone □ Decolorizer,
- Safranin □ Counter stain.

# PROCEDURE.

- Flood fixed smear with crystal violet stain for one minute.
- Wash slide with tap water.
- Flood it with Lugol's iodine for one minute.
- Wash it with clean tap water.
- Decolorize it for few (12) seconds with acetone / alcohol until no color is coming out with washing.

# Contd...

- Wash with tap water.
- Flood with safranin dye for one minute.
- Wash the stain with tap water.
- Keep the slide to air dry on a slide rack.
- Examine the smear microscopically.

# RESULT.

- Purple (blue) □ Gram Positive Bacteria.
- Pink (red) □ Gram Negative Bacteria.



**Gram Positive Bacteria**

**Gram Negative Bacteria**



